CHAPTER 2

ABNORMAL PLASMA NOREPINEPHRINE RESPONSE AND EXERCISE-INDUCED ALBUMINURIA IN IDDM PATIENTS

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Submaximal exercise provokes an abnormal elevation in albuminuria in patients with IDDM. Plasma catecholamines might be involved in this phenomenon by a renal vasoconstrictive effect. Twelve healthy subjects (Controls: albuminuria < 10µg/min), 13 normoalbuminuric IDDM patients (DNormo:albuminuria < 10µg/min) and 13 microalbuminuric IDDM patients (DMicro: albuminuria 10-200µg/min) performed a fixed bicycle workload (600 kpm for 20 min + urine collection 40 min postexercise). None of the patients suffered from autonomic neuropathy or hypertension. Fractional albumin clearance (FalbCl) rose in DNormo ($p = 0.02$) and DMicro ($p = 0.01$) but not in the Controls ($p = 0.40$). Basal plasma epinephrine and norepinephrine (NE) were not different in the three groups. The increments in NE were more pronounced in DNormo and DMicro than in Controls (Controls < Dnormo, $p < 0.05$; Controls < DMicro, $p < 0.01$). The changes in FalbCl were significantly correlated with the changes in NE (all subjects $r = 0.65$, $p < 0.001$). The increments in epinephrine were not different in the IDDM groups compared to the controls, and were not related to the changes in FalbCl. Multiple regression analysis showed that changes in plasma NE ($p < 0.002$) and in mean arterial pressure(MAP, $p < 0.005$) independently contributed to the changes in FalbCl (multiple $R = 0.73$).It is concluded that the exercise induced plasma NE response is increased in normo- and microalbuminuric IDDM patients. NE appears to contribute in the exercise-induced changes in renal protein handling, possibly by its effect on renal haemodynamics.

Introduction

Submaximal exercise has been reported to result in a marked elevation of albuminuria in patients with insulin-dependent diabetes mellitus (IDDM) with either normal or slightly elevated urinary albumin excretion at rest [1-7]. This albuminuric response is thought to be related to an increased glomerular passage of macromolecules, and possibly indicates an early impairment in the glomerular filtration barrier in IDDM [8]. Changes in tubular protein reabsorption are not considered to be of great importance during moderately strenuous exercise since the excretion of β2-microglobulin, a marker of tubular protein handling, does not increase under these circumstances [2,3].

The mechanisms by which exercise leads to a pronounced increase in albuminuria in IDDM patients are still poorly understood. During exercise the effective renal plasma flow (ERPF) decreases more than the glomerular filtration rate (GFR). Consequently, the filtration fraction i.e. the GFR divided by the ERPF increases [4,6,9]. A rise in the

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intraglomerular pressure, which is assumed to be reflected by this increase in filtration fraction [9], might be an important factor that contributes to the enhanced excretion of urinary albumin. An abnormal rise in systolic blood pressure has also been implicated in the exercise induced rise in albuminuria in IDDM particularly in conjunction with microalbuminuria [5], although this has not always been reported [2,6]. Studies in the rat have shown that norepinephrine (NE) increases renal vascular resistance predominantly via efferent glomerular vasoconstriction [10]. Both epinephrine and NE increase renal vascular resistance in humans [11-13]. It has therefore been proposed that the renal haemodynamic changes during exercise are related to increased circulatory levels of plasma catecholamines [9,14]. By a renal haemodynamic mechanism, plasma catecholamines thus could possibly affect renal protein handling during exercise in IDDM patients.

The purpose of the present study was to determine whether an altered plasma catecholamine response could play a role in the exaggerated rise in albuminuria in IDDM with normo- and microalbuminuria during moderately strenuous exercise.

Subjects and methods

Subjects

All subjects consented to the procedure after explanation of the purpose of the study, which was approved by the local medical ethics committee. Three groups of subjects were investigated (Table 1): 12 healthy control subjects (urinary albumin excretion rate (Ualb.V < 10 µg/min, Controls); 13 IDDM patients with normoalbuminuria (Ualb.V < 10 µg/min, DNormo), 13 IDDM patients with microalbuminuria (Ualb.V ranging from 10 to 200 µg/min, DMicro).

Ualb.V was determined in three consecutive overnight urine collections obtained within three months prior to the study. Three urine samples were used to classify the IDDM patients because of the high variability of Ualb.V [15]. A level of Ualb.V above 10 µg/min was considered to be abnormal (above the 97.5 percentile for 50 healthy controls subjects). Urinary tract infection was excluded by bacterial culture. All IDDM patients suffered from ketosis prone diabetes mellitus and were considered to be insulin-dependent on clinical grounds. All subjects had a serum creatinine level below 120 µmol/l and were normotensive (systolic blood pressure ≤140 mmHg and diastolic blood pressure ≤90 mmHg). No medication other than insulin or oral contraceptives were allowed. The IDDM patients did not have autonomic neuropathy as determined by Valsalva manoeuvre, beat to beat variation during deep breathing and blood pressure response to standing [16]. A questionnaire showed no differences in physical activity among the three groups. As is shown in Table 1 the three groups were closely comparable in age. There were no significant differences in sex distribution. The two IDDM groups were comparable with respect to duration of disease, metabolic control and retinopathy. All subjects were studied in the fasting state while the morning insulin dose was withheld. Blood pressure was measured using a sphygmomanometer (Baumano- meter, W.A. Braun Inc, N.Y., USA). Korotkoff phase 5 was taken as the diastolic blood pressure. Blood pressure and pulse rate were measured at 5 min intervals

during

Table 1. Clinical characteristics of the study groups.

	Controls (n=12)	DNormo (n=13)	DMicro (n=13)
Age (years)	25±3	26±6	27±5
Duration of disease (years)	--	10±4	12±5
Sex (males/females)	6/6	5/8	10/3
Ualb.V (µg/min)[a]	3.1 (1.6-7.8)	5.1(2.6-9.8)	21.3(10.2-166.7)[c]
Serum creatinine (µmol/l)	79±8	76±12	89±17[d]
Overnight creatinine clearance (ml/min per 1.73m^2)	109±27	110±29	102±23[d]
Body mass index (kg/m^2)	21.6±1.7	22.9±2.1	23.4±2.3
HbA_1 (%)	5.3±10.4[e]	7.7±1.6	7.6±1.2
Fasting blood glucose (mmol/l)	4.6±0.4[e]	11.9±4.7	12.1±5.2
Retinopathy (O/B/P)[b]	--	11/1/1	6/4/3
Systolic blood pressure change to standing (mmHg)	1 (-11 to 12)	3 (-12 to 15)	1 (-13 to 12)
Beat to beat variation (beats/min)	35±7	39±13	32±12
Valsalva ratio	1.76±0.28	1.72±0.28	1.78±0.32

Controls: control subjects; DNormo: IDDM patients with Ualb.V<10 µg/min; DMicro: IDDM patients with Ualb.V>10 µg/min and <200 µg/min. [a] Ualb.V: urinary albumin excretion rate; [b] Retinopathy: O: absent; B:background; P:proliferative. Values are given in mean±SD, except Ualb.V and systolic blood pressure change to standing which are given in median(range); [c] denotes $p<0.001$ from Controls, DNormo; [d] denotes $p<0.05$ from DNormo; [e] denotes $p<0.01$ from DNormo, DMicro.

the exercise and the recordings were averaged for analysis. Mean arterial pressure (MAP) was calculated as ⅔ diastolic + ⅓ systolic blood pressure.

Experimental design

The exercise protocol of Mogensen [1,2] was used, except that the subjects exercised only at 600 kpm for 20 min. The subjects drank 250 ml water per 20 min throughout the test from 0800 hours until 1200 hours to promote diuresis. Before the exercise the subjects were sitting for three hours. Urine was collected at 20 min intervals from 1000 hours until 1200 hours, including two postexercise collections. The exercise was performed on a bicycle ergometer from 1100 hours until 1120 hours. Blood samples were taken at regular intervals from a cannula inserted into an antecubital vein which was kept patent with a saline drip. During the study, the fractional clearance of albumin (FalbCl), calculated as the albumin clearance divided by the creatinine clearance, was used instead of the Ualb.V. This allowed us to take account of differences in GFR between the individuals and possible changes in GFR during the test, to correct for

changes in serum albumin concentration due to exercise induced haemoconcentration [17], and for possible errors in urine collection due to incomplete bladder emptying [15]. It was assumed that urinary albumin excretion had returned to baseline levels after two hours water loading [18] and so the 20 min urine collection directly before the exercise test was used as the reference period. FalbCl during the 20 min of exercise until 40 min thereafter was averaged for each subject to evaluate the effect of exercise.

Laboratory methods

Urine samples were stored at -20°C for a maximum of 2 weeks until analysis. Samples from each subject were determined in one run. Urinary albumin was measured using a commercially available double-antibody radioimmunoassay (Diagnostic Products Corporation, Apeldoorn, The Nether-lands, cat no KHAD2). The lower detection limit was 0.07 mg/l. Serum albumin was measured on a SMAC autoanalyzer (Technicon Instruments Inc. Tarrytown, NY, USA). Urinary and serum creatinine were measured on SMA(C) auto-analyzers. Blood glucose was measured on a Yellow Springs glucose analyser (Model 23A, Yellow Springs Inc., Yellow Springs, Ohio, USA). HbA1c was determined by colorimetry [19]. Plasma epinephrine and NE concentrations were measured using high-performance liquid chromotograhpy as previously described [20].

Statistical analysis

Results are expressed as mean ± SD for parametrically distributed data and as median (interquartile ranges) for non-parametrically distributed data. Comparisons of variables between groups were carried out using analysis of variance for parametrically and non- parametrically distributed data as appropriate. Changes of variables within groups were assessed by paired Student's t-tests or Wilcoxon tests. Adjustment for multiple comparisons was carried out using Duncan's method [21]. Differences in prevalence of clinical variables were analyzed by chi-square statistic. Correlations were sought using Spearman's rank correlation. Multiple regression analysis was used to disclose the independent contribution of parameters. *P*-values less than 0.05 were considered to be significant.

Results

Blood glucose

Blood glucose concentrations, measured directly before and after the exercise, were 4.5 ± 0.4 and 4.4 ± 0.6; 11.2 ± 3.1 and 11.7 ± 2.8; 11.1 ± 3.6 and 10.7 ± 4.2 mmol/l in Controls, DNormo and DMicro, respectively.

Blood pressure and pulse rate

Systolic and diastolic blood pressure were similar in the three groups at rest (Table 2). During exercise systolic blood pressure was higher in DMicro than in DNormo whereas diastolic blood pressure was lower in DNormo than in the other two groups. The increments in systolic blood pressure and MAP were larger in DMicro than in the other groups. The changes in Controls and in DNormo were similar. The pulse

rate was significantly higher in the two IDDM groups compared with the controls but the increment in response to exercise were comparable in all groups (Table 2).

Table 2. Blood pressure and pulse rate response to exercise.

	preexercise	during exercise	increase	
Bloodpressure (mmHg)	systolic/diastolic	systolic/diastolic	systolic	MAP
Controls (n=12)	122±11 / 79±8	154±16 / 73±7	32±8[a]	6±2[a]
DNormo (n=13)	118±10 / 73±6	150±17 / 66±9[c]	32±15[a]	6±5[a]
DMicro (n=13)	120±10 / 76±9	166±14[b] / 74±8	46±15[a,d]	15±9[a,d]
Pulse rate (beats/min)				
Controls (n=12)	67±11[e]	128±24[e]	61±19[a]	
DNormo (n=13)	79±9	150±21	71±22[a]	
DMicro (n-13)	80±10	145±13	69±12[a]	

Controls, DNormo, DMicro: see text and Table I. MAP: mean arterial pressure. Data are given in mean±SD. [a] denotes $p<0.001$ from preexercise; [b] denotes DMicro>DNormo, $p<0.05$; [c] denotes DNormo<Controls, DMicro, $p<0.05$; [d] denotes Dmicro>Controls, DNormo, $p<0.01$; [e] denotes Controls<DNormo, DMicro, $p<0.01$

Table 3. Effect of exercise on fractional albumin clearance.

Fractional albumin clearance ($\times 10^{-6}$)			exercise+postexercise
	preexercise	exercise+postexercise	% of prexercise
Controls (n=12)	0.69 (0.54-1.44)	0.82 (0.51-2.19)	111 (70-296)
DNormo (n=13)	0.75 (0.33-1.53)	2.04 (0.79-6.69)[a,e]	272 (87-1599)[e]
DMicro (n=13)	2.99 (1.77-11.97)[c,d]	10.93 (3.38-26.25)[b,d,f]	215 (104-524)[f,g]

Controls, DNormo, DMicro: see text and Table I. Data are given in median (interquartile ranges). [a] denotes DNormo>Controls, $p<0.05$; [b] denotes DMicro>DNormo, $p<0.05$; [c] denotes DMicro>DNormo, $p<0.001$; [d] denotes DMicro>Controls, $p<0.001$; [e] denotes $p=0.02$ from preexercise; [f] $p=0.01$ from preexercise; [g] % change in Dmicro>Controls, $p<0.05$.

Fractional albumin excretion

In the preexercise period no difference in FalbCl could be demonstrated between DNormo and Controls, but FalbCl was significantly higher in DNormo than in Controls during the exercise + postexercise period (Table 3). In DMicro, FalbCl was significantly higher compared with DNormo and Controls during both periods (Table 3). During the exercise + postexercise period, FalbCl increased in DNormo ($p = 0.02$) and in DMicro ($p = 0.01$) but not in Controls ($p = 0.40$). The relative change in FalbCl, expressed as a percentage of the preexercise values, was greater in DMicro than in Controls ($p < 0.05$).

Plasma norepinephrine and epinephrine concentrations

At baseline there were no differences in plasma epinephrine and NE concentrations among the three groups (Table 4). In all groups, plasma epinephrine and NE concentrations increased significantly in response to exercise. The exercise-induced increments in

Table 4. Plasma norepinephrine and epinephrine concentrations.

	preexercise	exercise		postexercise
	1000 hours	1100 hours	1120 hours	1200 hours
Norepinephrine (nmol/l)				
Controls (n=12)	2.3 (2.0-3.7)	2.5 (2.0-4.3)	3.5 (2.7-5.1)[a]	1.9 (1.2-2.5)
DNormo (n=13)	2.4 (1.4-3.1)	2.9 (1.9-3.3)	5.2 (3.7-6.7)[b,c]	2.3 (1.7-2.9)
DMicro (n=13)	2.1 (1.6-2.3)	2.2 (1.5-3.1)	5.8 (4.4-6.6)[b,d]	2.1 (1.6-2.4)
Epinephrine (nmol/l)				
Controls (n=12)	0.14 (0.08-0.21)	0.11 (0.09-0.18)	0.40 (0.20-0.50)[b]	0.16 (0.13-0.23)
DNormo (n=13)	0.13 (0.10-0.21)	0.12 (0.10-0.16)	0.30 (0.18-0.43)[b]	0.15 (0.06-0.25)
DMicro(n=13)	0.10 (0.08-0.26)	0.14 (0.07-0.29)	0.88 (0.43-0.97)[b,e]	0.18 (0.07-0.29)

Controls, DNormo, DMicro: see text and Table I. Data are given in median (interquartile ranges), [a] denotes $p<0.05$ and [b] denotes $p<0.01$ from preexercise; [c] denotes DNormo>Controls, $p<0.05$; [d] denotes DMicro>Controls, $p<0.02$; [e] denotes DMicro>Controls and DNormo, $p<0.05$.

plasma NE concentration were greater in both IDDM groups (DNormo: 2.5(1.0- 4.6) nmol/l; DMicro: 3.2(2.1-3.8) nmol/l) than in Controls (1.0(0.0-2.0) nmol/l) (Dnormo > Controls, $p < 0.05$; DMicro > Controls, $p < 0.01$) (Figure 1A). The exercise induced increments in plasma epinephrine were not different in Controls (0.24(0.11-0.35) nmol/l) compared with DNormo (0.18(0.08-0.29) nmol/l) and DMicro (0.52(0.22-0.70) nmol/l), but the increments in DMicro were greater than in DNormo ($p < 0.05$) (Figure 1B).

Relationships between plasma catecholamines, haemodynamics and glycaemia

The relative changes in FalbCl were positively related to the increase in plasma NE in all groups (All subjects $r = 0.65$, $p < 0.001$; Controls $r = 0.62$, $p < 0.05$; DNormo $r = 0.63$, $p < 0.05$; DMicro $r = 0.56$, $p < 0.05$) (Figure 2). The exercise induced changes in FalbCl were also correlated with the increase in MAP in DNormo ($r = 0.64$, $p < 0.01$) and in DMicro ($r = 0.60$, $p < 0.02$) but not in Controls ($r = 0.25$, $p = 0.22$). Multiple regression analysis demonstrated that both changes in plasma NE ($p < 0.002$) and in MAP ($p < 0.005$) independently contributed to the exercise induced alterations in FalbCl (multiple $r = 0.73$, $n = 38$). Multiple regression analysis in two IDDM groups showed similar independent effects of plasma NE ($p < 0.02$) and MAP ($p < 0.005$) on the changes in FalbCl (multiple $r = 0.75$, $n = 26$). When multiple regression analysis was carried out using the increments in systolic blood pressure instead of MAP the contribution of blood pressure rise was not significant ($p = 0.20$).

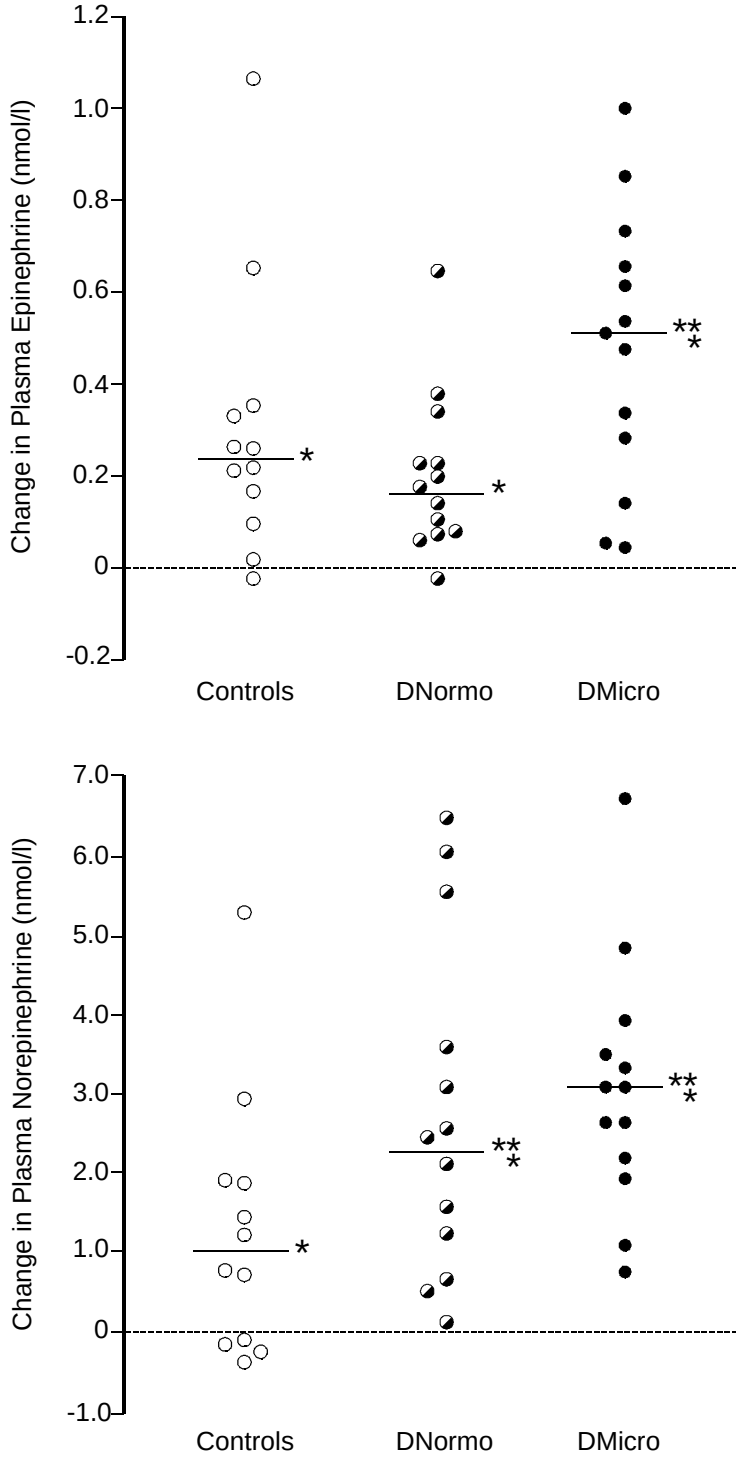

Figure 1. Changes in plasma norepinephrine and epinephrine concentrations in response to exercise. Controls ○, DNormo ○, DMicro ●: see text and Table 1. Bars are median values. Upper panel: Increments in plasma epinephrine concentration. * denotes $p < 0.01$ from preexercise; ** denotes increment in DMicro > DNormo, $p < 0.05$. Lower panel: Increments in plasma norepinephrine concentration. * denotes $p < < 0.05$ from preexercise; ** denotes increment in DNormo and Dmicro > Controls, $p < < 0.05$.

Baseline plasma NE concentrations and pulse rate were not correlated. During exercise a positive relationship was observed between plasma NE and pulse rate in Controls ($r = 0.77$, $p < 0.01$) as well in the combined IDDM groups ($r = 0.53$, $p < 0.01$). No significant correlations were found between plasma epinephrine concentrations and either FalbCl, pulse rate or blood pressure during exercise in any of the groups. In the IDDM groups the changes in plasma epinephrine and NE concentrations were not related to actual glycaemia and HbA1.

Discussion

Exercise has been proposed to provoke abnormalities in renal protein handling in IDDM [1,2]. It is unclear whether plasma catecholamines are involved in this abnormal rise in albuminuria. The subjects participating in the present study did not have systemic hypertension or signs of autonomic neuropathy. Patients with an abnormal urinary albumin excretion rate above 10 µg/min were designated microalbuminuric but it should be noted that levels higher than 20 µg/min are considered to represent a risk marker for diabetic nephropathy [22].

Exercise induced an abnormal rise in albuminuria in both IDDM groups in accordance with many previous studies employing a comparable fixed workload [1-5]. The mechanisms which are responsible for this abnormal rise in albuminuria are still not precisely understood. Systemic blood pressure rise, alterations in renal haemodynamics and in glomerular perm selectivity or a combination of these factors are likely to be involved [1-9]. As earlier studies have shown [2,6] no difference in blood pressure response was observed between the normoalbuminuric IDDM and non-diabetic subjects, making it unlikely that an abnormal increase in blood pressure per se was responsible for the marked albuminuric response in this group of patients. In the microalbuminuric IDDM subjects, the increase in blood pressure was larger than in the other groups, in accordance with other data [5], suggesting that alterations in systemic haemodynamics could have contributed to the abnormal rise in albuminuria in these patients. This study showed a significant relationship between the increase in plasma NE and the albuminuric response during exercise. In addition, the rise in plasma NE was significantly greater in normo- and microalbuminuric IDDM patients than in the normal subjects, although there was a considerable overlap in individual responses. Multiple regression analysis substantiated that both the exercise induced rise in plasma NE and in MAP had an independent effect on the albuminuric response. It is noteworthy that, although used by some investigators [7], diastolic blood pressure recordings are probably underestimated when measured by the auscultatory method during exercise [23]. Several studies have shown a relationship between (change in) systolic blood pressure and albuminuria during exercise [3,5], but this has not consistently been reported [4,6]. In this investigation, excluding patients with hypertension, the contributory effect of systemic blood pressure was not significant when using systolic blood pressure instead of MAP in the multiple regression analysis.

It has been documented that the filtration fraction rises more and is higher during exercise in IDDM subjects, especially in patients with microalbuminuria [4,6]. It is therefore probable that there is a link between the increased glomerular passage of

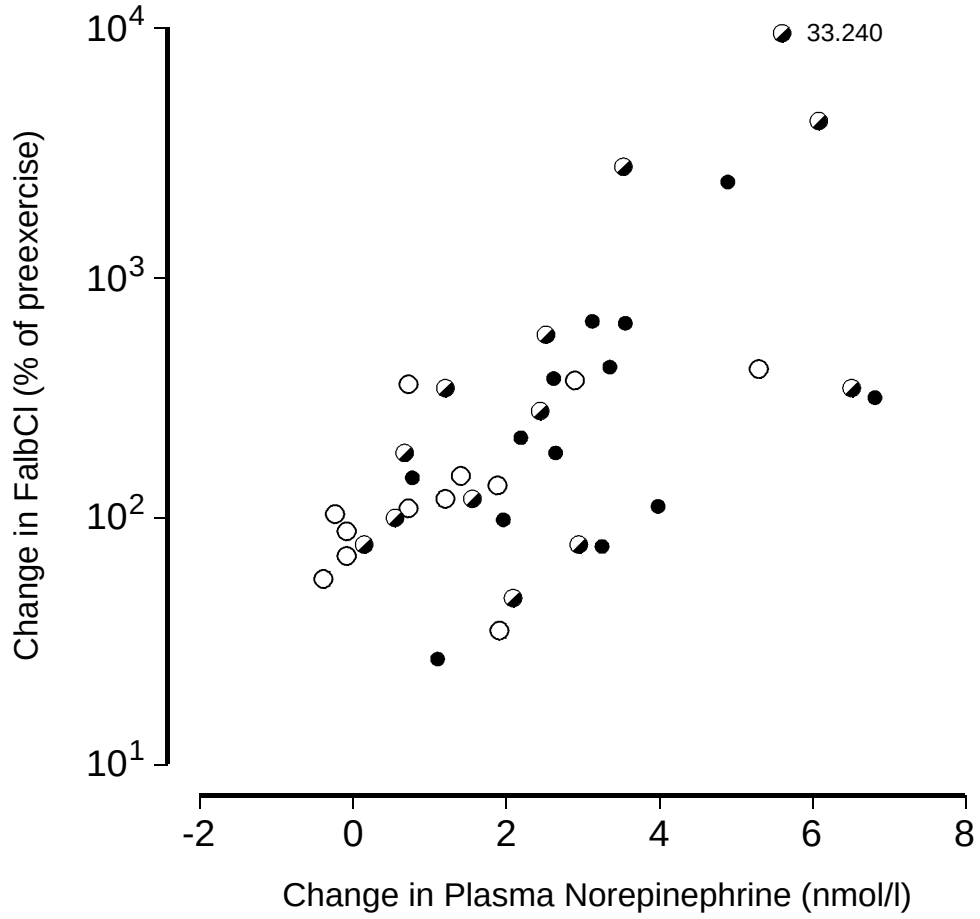

Figure 2. Changes in plasma norepinephrine concentration and relative changes in fractional albumin clearance in response to exercise. Controls ○, DNormo ○, Dmicro •: see text and Table 1. Relative changes in fractional albumin clearance are expressed as % of pre-exercise values. All subjects (n = 38):$r = 0.65$, $p < 0.001$; Controls (n = 12) ○:$r = 0.62$, $p < 0.05$ DNormo (n = 13) ○:$r = 0.63$, $p < 0.05$; DMicro (n = 13) ●:$r = 0.56$, $p < 0.05$

albumin and the rise in filtration fraction during exercise in IDDM patients. Experimental studies in the rat have shown that infusion of NE results in an increase in filtration fraction in parallel with directly measured increments in intraglomerular pressure [10], whereas the degree of albuminuria in response to NE is closely related to changes in filtration fraction [24]. Taken together, the previously reported renal haemodynamic changes [4,6] and the presently shown significant relationship between changes in albuminuria and changes in plasma NE concentrations during exercise would support the hypothesis that NE is involved in the increase in albuminuria during exercise [9,14]. This present observations also suggest that an altered catecholamine response could possibly contribute to an abnormal rise in albuminuria in IDDM.

Several factors might be involved in the abnormal rise in plasma NE during exercise in IDDM. A fixed workload was employed in the expectation that this would

discriminate IDDM and non-diabetic subjects with respect to their albuminuric response [1-5]. It has been suggested that the maximal working capacity is reduced in microalbuminuric but not significantly so in normoalbuminuric IDDM patients [6]. Aerobic working capacity was found to be decreased in patients with autonomic neuropathy [17,25] and microalbuminuria whereas normoalbuminuric patients showed a normal maximal oxygen uptake [26]. It seems therefore probable that the fixed workload was more stressful for the microalbuminuric patients but it is unlikely that the relative workload was larger for the normoalbuminuric IDDM patients. In addition, it has been shown that an exaggerated catecholamine response can be related to suboptimal metabolic control [27,28], but no relationship between plasma catecholamines and HbA1c or actual glycaemia could be demonstrated in this study. The expected increase in pulse rate [2,7] raises the possibility of an abnormal regulation of the autonomic nervous system in IDDM [29]. Moreover, an enhanced vasopressor responsiveness to infusion of NE has been demonstrated in IDDM patients either without overt microvascular complications [30] or with microalbuminuria [31]. Whether glomerular vascular structures exhibit such an increased sensitivity to circulatory catecholamines remains to be elucidated.

In conclusion, the present observations suggest that renal protein handling during exercise is related to both circulating plasma NE and blood pressure in IDDM. The exaggerated albuminuric response, even in the absence of an increased urinary albumin excretion at rest, was associated with abnormalities in systemic catecholamine regulation. Direct experiments are needed to demonstrate a causal relationship between these phenomena. In addition, the rise in plasma NE was significantly greater in normo- and microalbuminuric diabetic patients than in the normal subjects, although there was a considerable overlap in individual responses.

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